The use of the computer technology for the evaluation of the strict morphological sperm analysis

Z. Věžník, O. Matoušková, D. Švecová, A. Zajícová

Veterinary Research Institute, Brno, Czech Republic

ABSTRACT: A programme for evaluating the strict morphological analysis of sperm was developed. The programme was verified by conducting 552 morphological analyses of bull, stallion, boar and human ejaculates. The method was evaluated by comparing the results obtained by routinely used morphological examinations with the results received by the automatic method SASMO. The following advantages of the programme were demonstrated: it is possible to obtain information on the proportion of normal and pathological spermatozoa, to determine the frequency of the respective characteristics analysed, to express detected alterations per pathological spermatozoon (teratosperm index), to express frequency of respective alterations per total number of spermatozoa examined, to display alterations according to their frequency and differentiate between developmental and acquired alterations. It was proved that using the detailed morphological evaluation of sperm by the survival test, it is possible to get statistically significant information on the prediction of sperm survival. The programme represents an important aid for making the morphological evaluation of sperm quality more objective both in veterinary and human medicine and in all insemination stations and assisted reproduction centres.

Keywords: bull; stallion; boar; human; diagnosis; spermatology; morphology; strict method

INTRODUCTION

World literature repeatedly demonstrates the significant role of morphological ejaculate examination for evaluating its quality level (Johnson, 1997; Johnson *et al*., 1998; Ombelet *et al*., 1995, 1997; Host *et al*., 1999; Boersma and Braun, 1999).

Not all sperm produced are normally developed. This state, which occurs even under optimum conditions, is influenced by a whole range of internal as well as external environmental factors. The fact that most ejaculate is used for the preparation of insemination doses, increases the significance and need for the morphological examination of ejaculates of breeding animals which are included in such practice. Many authors have studied the evaluation and classification of changes in sperm (Hofmann *et al*., 1985; Johnson, 1997; Gravance *et al*., 1998, 1999; Boersma and Braun, 1999; Menkveld a Kruger, 1995; Check *et al*., 1992). Different views about the significance of individual changes in sperm are still being discussed and in many cases it is impossible to unambiguously judge their importance even today. In agreement with Eliasson (1971, 1981) as well as the WHO manual (1992) and considering the multiparametric recording system, teratosperm index, it is possible to comply with the idea that whole sperm must be considered during evaluation. Jouannet *et al*. (1988) presented the relationship between the multiple anomaly index and fertilisation potency.

In the course of sixty years, the criteria and evaluation methods of sperm morphology have witnessed a whole range of turns of opinion. In accordance with world literature, the role of morphological sperm evaluation is one of the foremost predictors of fertilisation. Sperm morphology has the advantage of accuracy and precision of evaluation, is straightforward and, as far as the equipment needed is concerned, is accessible for most laboratories.

MATERIAL AND METHODS

In order to list the criteria for evaluating changes in sperm, 76 bull ejaculates, 156 boar, 29 stallion and 30 man ejaculates were examined in a laboratory for the diagnosis of fertility disorders. All ejaculates were examined using a dynamic test at an original value and during a 120-minute short-term survival test. In total, 552 spermanalyses were evaluated.

Through the analysis and assessment of the morphological findings of these ejaculates, 36 basic criteria were set to develop a programme for evaluating the morphological changes in sperm using the strict method.

Supported by the Ministry of Agriculture of the Czech Republic (Grant from National Agency for Agricultural Research in Prague, No. EP 9197).

Table 1. The frequency of findings of changes in sperm using the SASMO .method

Criteria		Ejaculates of							
		bulls (n = 76)		stallions (n = 29)		boars (n = 156)		men (n = 30)	
		mean	SD	mean	SD	mean	SD	mean	SD
Head									
macrocephalia	e	0.9	0.91	0.2	0.44	0.56	0.58	3.23	2.29
microcephalia	e	1.11	1.02	2.67	2.02	0.74	1	7.01	5.29
narrowed elongated	e	4.33	3.69	1.97	1.67	1.18	1.23	7.78	7.353
pear-shaped (pyriform)	e	1.71	2.27	1.92	1.46	0.6	1.24	4	2.94
teratoid (amorphous)	e	0.81	1.11	1.4	1.54	0.02	0.09	15.6	7.19
abortive (pycnotic)	e	0.32	0.46	1.1	1.32	0	0	5.19	4.97
diadem defect	e	0.01	0.07	0	0	0	0	0	0
comb-shaped head (crista)/	e	0.27	0.38	0.15	0.43	0	0	0	0
doubled head	e	0.04	0.17	0.17	0.41	0.17	0.35	0.3	0.59
persistant acroblast	e	0.31	0.59	1.17	1.23	0.19	0.35	0.03	0.18
condensation of acrosome	e	0.18	0.31	0.017	0.09	0.16	0.42	0	0
swelling of acrosome	a	12.59	6.62	3.36	2.56	11.39	4.36	0.03	0.18
release of acrosome	a	0.81	0.9	0.15	0.72	0.18	0.32	0	0
without acrosome	a	2.39	3.55	4.41	3.58	0.44	1.95	0.1	0.39
vacuolization within acrosome	a	0.08	0.27	0.03	0.18	0.11	0.28	7.97	6.76
marbling	a	0.15	0.37	0	0	0	0	0	0
obscure aquatorial segment	a	0.12	0.46	0	0	0.04	0.16	0	0
changed base of head	e	4.71	2.95	0.6	0.9	1.37	1.21	2.23	3.27
Neck									
abnormal position of tail insertion	e	0.93	0.95	1.2	1.11	0.36	0.59	3.44	3.26
loosening of bond in implantal groove	e	0.21	0.35	0	0	0.01	0.07	0	0
cytoplasm residuals in neck area	e	0.53	0.57	2.56	1.87	3.71	3.93	2.04	2.05
Midpiece									
thickened	e	2.5	1.91	0.84	0.9	0.3	0.55	0	0
narrowed	e	0.06	0.19	0	0	0.04	0.13	0	0
changed length	e	0.05	0.18	0	0	0	0	0	0
loosening of mitochondrial spiral	e	0.34	0.83	0	0	0.06	0.19	0	0
partial absence of mitochondrial spiral	e	0.15	0.27	1.16	0.35	0	0	0.07	0.36
spiral shape	e	0.12	0.24	0	0	0.01	0.07	0	0
pseudodroplet	e	0.08	0.25	0	0	0.01	0.07	0	0
doubling	e	0.03	0.12	0.03	0.18	0	0	0	0
Tail									
bent tail	a	1.88	2.44	7.59	6.95	2.02	3.74	0.73	1.03
coiled tail	a	0.33	0.61	2.17	3.22	0	0	2.07	1.63
convolution tail	a	0.55	0.94	0.79	1.06	0.11	0.23	0.17	0.52
primary tail convolution-Dag defect	e	1.73	2.34	1.92	1.67	0.5	0.64	9.34	7.53
agenesis-hypogenesis	e	0.22	0.39	2.33	2.09	0.01	0.07	1.1	1.81
broken tail	a	0.15	0.32	0.22	0.47	0.04	0.13	0.13	0.43
doubling tail	e	0.14	0.32	0.12	0.36	0.07	0.2	1.27	1.59

e = evolutionary
a = acquired

The set of criteria for multiparametric evaluation has the prevalence of evolutionary (primary) changes over the acquired (secondary) changes (25 : 11) – Table 1. The only changes referred to as acquired are those which could have come into existence after the definite end of structural development of the sperm. Despite this, such changes represent significant information about the resistance of sperm membrane structures.

The basis of the analysis is the evaluation of 200 cells in a smear stained using a method described by Hancock (1959).

Following fixation using a solution described by Hancock for 30 minutes, preparations were hold in a staining mixture for 16 hours and after rinsing they were left to air-dry.

A computer programme SASMO (Strict Analysis of Sperm Morphology) was developed to enable the automation of morphological analysis evaluation.

The data obtained are expressed by:

- the number of normal sperm and pathologically changed sperm,
- setting the frequency of findings within individual criteria,
- conversion of fixed changes per one pathological sperm i.e. expressing the index of teratosperm,
- the frequency of individual changes per number of all evaluated sperm,
- the frequency of individual changes per number of pathological sperm,
- listing changes according to their frequency,
- the classification of changes according to their genesis to evolutionary and acquired.

The results of the strict analysis were compared with routinely used summaries of morphological changes in sperm in spermatological labs.

All analyses of the morphological picture were used to establish the frequency of changes in individual criteria of the SASMO morphological analysis.

The correspondence of results of the examination carried out by two evaluators was analysed with the ejaculates of 49 bulls from 3 insemination stations; these were evaluated using routine classification as well as the method of strict analysis.

65 analyses of bull, 91 boar, 29 stallion and 30 man ejaculates were included in establishing the mean teratosperm indices for the individual species of farm animals and the man. A histogram of teratosperm index values was established and a cumulative frequency of values up to the mean was set for all analyses.

49 bull ejaculates evaluated using the strict analysis were used to establish the frequency of individual changes in the sperm of bull.

29 stallion and 35 boar ejaculates which had been statistically proven to be related were analysed in order to express the relation of the teratosperm index to the increase of surface changes in sperm. In order to prove sperm resistance we used a comparison of 22 survival tests of boar semen (a 120-minute survival test) and sperm in an insemination dose stored at 16°C for 72 hours.

As statistically evaluated data does not meet the prerequisite of normality of spreading, methods of non-parametric tests module of the STAT Plus programme (Matoušková *et al*., 1992) were used to process results statistically.

RESULTS

Table 1 outlines the frequency of findings within the established criteria for the strict analysis of morphological changes in the sperm of bulls, stallions, boars and men. The results prove the usability of the given criteria for expressing a morphological graph of the ejaculate.

Values obtained through examining 49 bull ejaculates by evaluators A and B are presented in Table 2.

Table 2. A summary of findings of morphological examinations in sperm obtained by two evaluators (bull ejaculates, n = 49)

Number of findings	A	B
Mean	40.84	40.69
SD	14.12	13.51
Minimum	13	14
Maximum	95	93
Percentage of pathological sperm	**A**	**B**
Mean	31.6	33.9
SD	8.9	8.7
Minimum	12	17
Maximum	56	58

The correspondence between the examinations is confirmed by the highly significant Spearman coeficients of the order correlation 0.848 found for the numbers of findings and 0.905 for the percentage of pathological sperm.

Table 3 demonstrate morphological analyses of semen of the same group of bulls carried out using a routine evaluation method and the strict method.

The higher ratio of pathological sperm in the analyses carried out using the method of multiparametric recording proves the suitability of this method for a more accurate characterisation of the morphological changes and is linked to the teratosperm index.

The teratosperm index which expresses the frequency of malformations in sperm considered as abnormal was established for representatives of the main species of breeding stock and the man (Table 4).

Statistically highly significant correlation coeficient 0.319 (p = 0.01) was obtained by comparing the size of

Table 3. Morphological analyses carried out using a routine method of evaluation and the strict method (bull ejaculates, $n = 49$)

Routine classification		Criteria of strict analysis	
Pathological findings	27.3%	Pathological findings	33.1%
SD	9.5	SD	8.9

Correlation coeficient 0.583 is statistically highly significant ($p = 0.01$)

Routine classification		Criteria of strict analysis	
Evolutionary – primary changes	13.04%	Evolutionary – primary changes	17.51%
SD	5.07	SD	5. 91

Correlation coeficient 0.524 is statistically highly significant ($p = 0.01$)

Routine classification		Criteria of strict analysis	
Acquired – secondary changes	14.46%	Acquired – secondary changes	15.59%
SD	7.59	SD	6.59

Correlation coeficient 0.422 is statistically highly significant ($p = 0.01$)

Table 4. Teratosperm index of main species of farm animals and the man

Species	*n*	Mean	SD	Frequency (%)	Maximum
Bull	65	1.235	0.16	76	1.8
Boar	91	1.141	0.09	86	1.5
Stallion	29	1.329	0.11	79	1.5
Man	30	1.345	0.12	79	1.6

teratosperm index and the total number of established malformations.

From the list of most frequent findings (Table 5), it is clear that acquired changes dominate over evolutionary changes (the first ten findings show the frequency ratio of 22.85% of acquired and 17.64% of evolutionary changes). The swelling of the acrosome has proven to be a significant factor of sperm membrane system resistance during short-term as well as long-term survival tests. In 29 stallion ejaculates, for example, the frequency of findings within this criterion increased on average by 3.9% (SD 4.26, min. 4.1, max. 12.8) with 120-minute survival rate. A similar finding was established in 35 analysed boar ejaculates from the ISK production. The increase of swollen acrosomes came to 8.4% on average when compared to the initial value (SD 4.5, min. 1.5, max. 19.5).

The rise of secondary changes represented by the swelling of acrosomes was correlated with a different teratosperm index value in the initial value and during a 120-minute survival test. In the 35 boar ejaculates examined, the correlation coeficient –0.572 was highly statistically significant ($p = 0.05$) with the positive value of 0.422. The appearance of such difference is proven by the magnitude of relative ratios of the increase the total number of pathological sperm in stallions by 21.1% and in boars by 41.3%.

The indicator of the increase of swollen acrosomes for significant individuality of separate ejaculates, as understood from the variable span, was used to compare with the indicators of sperm survival in an insemination dose. In 22 boar ejaculates, an increase was established in the swelling of acrosomes during 120 minutes of a survival test and compared with sperm survival and the increase of morphological changes in sperm in an insemination dose after 72 hours. The correlation coefficient which proves the correspondence between the indicators of the resistance of sperm membrane structures was staistically significant 0.482 ($p = 0.05$).

DISCUSSION

The aim of this experimental study is to express the diagnostic meaning of strict analysis of sperm morphology. By means of a developed and validated programme for automatic evaluation of morphological analyses (SASMO), results of 200 sperm evaluations are obtained in a setting which is provided by the programme and which significantly increases the accuracy and speed of interpretation of an ejaculate. Making a smear and staining it remains the basis of the analysis. The standardisation of this step in the method is necessary for every laboratory without exception if results are to be comparable. The contribution of the method becomes relevant and significant particularly following the statement of Steigerwald and Krause (1998) who tested the automatic system for determining pathological sperm according to WHO methodology (1992). Their study proved that the time which was needed for examination by means of the automatic system for the determination of the morphological changes in sperm was twice as long as the time

Table 5. Frequency of findings during multiparametric sperm morphology (bull ejaculates, $n = 65$)

Criterium	%	
Swelling of acrosome	12.59	a
Changed base of head	4.91	e
Head narrowed elongated	4.52	e
Head without acrosome	2.74	a
Connecting part thickened	2.66	e
Bent tail	2.24	a
Head pear-shaped (pyriform)	2.15	e
Primary tail convolution-Dag defect	1.89	e
Microcephalia	1.51	e
Release of acrosome	1.27	a
Macrocephalia	1.22	e
Loosening of mitochondrial spiral	1.18	e
Abnormal position of tail insertion	1.17	e
Teratoid head (amorphous)	1.09	e
Convolution of tail	1.04	a
Obscure aquatorial segment	1	a
Persistant acroblast	0.93	e
Coiled tail	0.89	a
Cytoplasm residuals in neck area	0.87	e
Vacuolization within acrosome	0.8	a
Pseudodroplet	0.8	e
Doubling of tail	0.78	e
Abortive head (pycnotic)	0.77	e
Marbling	0.75	a
Notching of tail	0.75	a
Agenesis-hypogenesis of tail	0.73	e
Doubled head	0.67	e
Loosening of bond in implant. Groove	0.66	a
Comb-shaped head (crista)	0.65	e
Condensation of acrosome	0.64	e
Changed length of midpiece part	0.62	e
Narrowed connecting part	–0.6	e
Partial absence of mitochondrial spiral	0.58	e
Spiral shape of midpiece part	0.54	e
Doubling of mitpiece part	–0.5	e
Diadem defect	–0.5	e

e = evolutionary changes (primary)
a = acquired changes (secondary)

needed for direct microscopic evaluation. The basic equipment needed for using the SASMO method is a microscope correspondingly equipped for cytomorphology. A computer is needed for the programme application. The methodology of evaluation consists in determining morphologically normal sperm (n) and pathologically changed sperm (p). Every abnormality indicates a pathologically changed sperm and the programme does not allow further evaluation, unless change or changes are defined according to the set of established criteria. By offering 36 criteria, the programme facilitates precise categorisation. An independent component of the evidence is formed by detached heads (a), detached tails (b) and proximal droplet (c). These values are presented separately because of their specific diagnostic position.

The frequency of individual changes is expressed as a percentage of the total number of sperm examined (including the normo) and a percentage of the number of pathological sperm. On the basis of this last analysis it is possible to compile a list of changes according to their frequency. This overview permits orientation regarding the nature of the changes and their importance, both from the perspective of frequency and from the perspective of their genesis.

The classification of changes creating a group of primary, evolutionary abnormalities and of changes of a secondary, acquired nature presents the distribution of the whole collection of findings according to fixed criteria. Because of the dominance of criteria inhibiting evolutionary changes and of the SASMO principle, in which more findings may be recorded upon each sperm, the percentage of primary changes is always higher than under the classic form of assessment. Also the total number of pathologically changed sperm is higher than under the classic method of evaluation and produces a marked share of abnormalities, which under the classic method are not indicated.

The list showing the frequency of individual findings on sperm creates a basic database, from which a teratosperm index is calculated, which constitutes the average number of all abnormalities per single pathosperm.

The size of the teratosperm index defines the ratio of findings to the number of pathological sperm and its changes in a survival test express the morphological resistance of the sperm.

In 1970 a test for the morphological resistance of sperm was proposed (Věžník, 1970), expressing the individual growth of surface changes on sperm in the course of survival of a 120 minute test as one of the indicators of the quality of the ejaculate. Changes of the teratosperm index broaden the interpretation about the stability of the integrity of the membranes of normosperm. Despite the currently increasing role of surface changes on sperm and on the teratosperm index, the ratio of the normo and pathosperm is not markedly changed. If these changes increase when the teratosperm index is reduced, the ratio of pathological sperm as a sign of low quality ejaculate increases at the same time.

The use of the SASMO programme produces an overview protocol about the examination of semen, the possibility of a detailed analysis for the interpretation of a finding, a summary of all abnormalities fixed on the sperm and an order of frequency of fixed changes as a guide to expressing the significance of the finding.

The programme is a significant aid for materilizing the morphological evaluation of the quality of ejaculates in

both veterinary and human medicine and in all insemination processes and processes of assisted reproduction.

REFERENCES

Boersma A., Braun J. (1999): Computer assisted analysis of sperm morphology in veterinary medicine. Berl. Munch. Tierarztl. Wochenschr., *112*, 81–85.

Check J.H., Bollendorf A., Press M., Blue T. (1992): Standart sperm morphology as a predictor of male fertility potential. Arch. Androl., *28*, 39–41.

Eliasson R. (1971): Standards for investigation of human semen? Andrologie, *3*, 49–64.

Eliasson R. (1981): Analysis of sperm. In: Burger H., De Kretser D.: The Testis. Raven Press, New York. 381–399.

Gravance C.G., Vishwanath R., Pitt C., Garner D.L., Casey P.J. (1998): Effects of cryopreservation on bull sperm head morphometry. J. Androl., *19*, 704–709.

Gravance C.G., Garner D.L., Pitt C., Vishwanath R., Sax-Gravance S.K., Casey P.J. (1999): Replicate and technician variation associated with computer aided bull sperm head morphometry analysis (ASMA). Department of Obstetric and Gynaecology, University of Auckland, New Zealand, Int. J. Androl., *22*, 77–82.

Hancock J.L. (1959): The morphologic characteristics of spermatozoa and fertility. Int. J. Fertil., *4*, 347–359.

Hofmann N., Freundl G., Florack M. (1985): Die Formstörungen der Spermatozoen im Sperma und Zervikalschleim als Spiegel testekulärer Erkrankungen. Gynäkologie, *18*, 191–197.

Host E., Lindenberg S., Ernst E., Christensen F. (1999): Sperm morphology and IVF: embryo quality in relation to sperm morphology following the WHO and Kruger40s strict criteria. Acta Obstet. Gynecol. Scand., *78*, 526–529.

Johnson W.H. (1997): The significance to bull fertility of morphologically abnormal sperm. Bull Infertil., *13*, 255–270.

Johnson K.R., Dewey C.E., Bobo J.K., Kelling C.L., Lunstra D.D. (1998): Prevalence of morphologic defects in spermatozoa from beef bulls. JAVMA, *213*, 1468–1471.

Jouannet P., Ducot B., Feneux X., Spira A. (1988): Male factors and the likehood of pregnancy in infertile couples. 1.Study of sperm characteristics. Int. J. Androl., *11*, 379–394.

Matoušková O., Cígler M., Chalupa J., Hruška K. (1992): Manual of the Programme STAT-Plus. Veterinary Research Institute, Brno. 168 pp.

Menkveld R., Kruger T.F. (1995): Advantages of strict (Tygerberg) criteria for evaluation of sperm morphology. Int. J. Androl., *18*, 36–42.

Ombelet W., Menkvelt R., Kruger T., Steeno O. (1995): Sperm morphology assessment: historical review in relation to fertility. Hum. Reprod. Update, *1*, 543–557.

Ombelet W., Wouters E., Boels L., Cox A., Janssen M., Spiessens C., Vereecken A., Bosmans E., Steeno O. (1997): Sperm morphology assessment: diagnostic potential and comparative analysis of strict or WHO criteria in a fertile and a subfertile population. Int. J. Androl., *20*, 367–372.

Steigerwald P., Krause W. (1998): Estimation of sperm morphology using a new CASA system. Andrologia, *30*, 23–27.

Věžník Z. (1970): Utilization of morphological Indices for a qualitative evaluation of ejaculates (in Czech). Vet. Med. (Praha), *15*, 571–578.

WHO (1992): Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction. Cambridge University Press, Cambridge.

Received: 01–01–12
Accepted after corrections: 01–03–26

Corresponding Author:

Prof. MVDr. Zdeněk Věžník, DrSc., Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel. +420 5 41 32 12 41, fax +420 5 41 21 12 29, e-mail: veznik@vri.cz